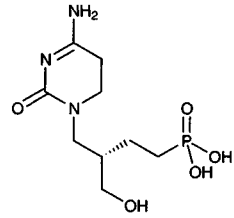
Cidofovir



Molecular formula: $C_8H_{14}N_3O_6P$
Molecular weight: 279.19
CAS Registry No.: 113852-37-2
Merck Index: 2329

SAMPLE

Matrix: blood
Sample preparation: Briefly vortex 100 µL plasma with 300 µL 500 ng/mL IS in MeCN: water:acetic acid 80:19:1, centrifuge at 15000 g for 5 min. Remove the supernatant and add it to 100 µL 1.25 mM phenacyl bromide in MeCN, heat at 80° for 45 min, evaporate to dryness under reduced pressure at room temperature, reconstitute with 60 µL water, vortex briefly, centrifuge at 15000 g for 5 min, inject a 20 µL aliquot of the supernatant.

HPLC VARIABLES

Column: 100 × 4.6 5 µm Prodigy ODS-2 (Phenomenex)
Mobile phase: MeCN:water 30:70 containing 12 mM phosphoric acid and 6 mM dodecyltriethylammonium phosphate (Q12) (Bodman) (final pH 3.0-3.1)
Column temperature: 45
Flow rate: 3
Injection volume: 20
Detector: F ex 305 em 370

CHROMATOGRAM

Retention time: 3.1
Internal standard: cytidine-5'-monophosphate (5.3)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Noninterfering: lamivudine, zalcitabine

KEY WORDS

derivatization; monkey; plasma

REFERENCE

Eisenberg,E.J.; Cundy,K.C. High-performance liquid chromatographic determination of cytosine-containing compounds by precolumn fluorescence derivatization with phenacyl bromide: application to antiviral nucleosides and nucleotides, *J.Chromatogr.B*, **1996**, *679*, 119–127.

SAMPLE

Matrix: dialysate

HPLC VARIABLES

Column: C18
Mobile phase: MeCN:MeOH:2.5 mM pH 3.0 ammonium dihydrogen phosphate buffer 17.5: 17.5:65
Detector: UV 278

CHROMATOGRAM

Retention time: 2.5
Internal standard: tryptophan (4.2)
Limit of detection: 10 ng/mL

KEY WORDS

pharmacokinetics; rabbit; microdialysis

REFERENCE

Duggirala,S.M.; Mitra,A.K. Intravitreal pharmacokinetics of anti CMV agents ganciclovir and cidofovir -a comparison (Abstract 1119), *Pharm.Res.*, **1997**, *14*, S39–S39.

SAMPLE

Matrix: formulations
Sample preparation: If necessary, dilute injection 1:50 with water, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 4.6 (Alltech Associates S/N 94091346)
Column: 150 × 4.6 5 μm Hypersil octadecylsilane C18
Mobile phase: 3.5 mM Na_2HPO_4 containing 5 mM tetrabutylammonium dihydrogen phosphate, adjusted to pH 6.0 with concentrated phosphoric acid
Flow rate: 2
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 7

OTHER SUBSTANCES

Simultaneous: degradation products

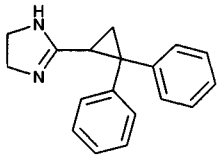
KEY WORDS

5 % dextrose; 0.9 % sodium chloride; injections; stability-indicating

REFERENCE

Yuan,L.-C.; Samuels,G.J.; Visor,G.C. Stability of cidofovir in 0.9% sodium chloride injection and in 5% dextrose injection, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 1939–1943.

Cifenline



Molecular formula: $C_{18}H_{18}N_2$
Molecular weight: 262.35
CAS Registry No.: 53267-01-9, 100678-32-8 (succinate)
Merck Index: 2330
Lednicer No.: 4 87

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 250 μL triethylamine + 100 μL 1 M NaOH + 100 μL 15 μg/mL p-chlorodisopyramide in 10 mM HCl + 1.75 mL dichloromethane, rotate slowly for 20 min, centrifuge at 3600 g for 20 min. Remove 1 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 500 μL 10 mM HCl, inject a 200 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.6 5 μm Nucleosil CN
Mobile phase: MeCN:buffer 35:65 (Buffer was 25 mL PIC B-8 (1-octanesulfonic acid), 2.5 mL PIC D-4 (dibutylamine phosphate), 1 mL butylamine, and 971.5 mL water.)
Flow rate: 1.1
Injection volume: 200
Detector: UV 214

CHROMATOGRAM

Retention time: 11.5
Internal standard: p-chlorodisopyramide (18.5)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: aspirin, betaxolol, diazepam, digitoxin, dihydralazine, enalapril, furosemide, glibenclamide, heparin, hydrochlorothiazide, isosorbide mononitrate, metildigoxin, metoprolol, nifedipine, nitrendipine, phenprocoumon, ranitidine, spironolactone, triamterene, verapamil, xipamide

KEY WORDS

serum

REFERENCE

Kühlkamp,V.; Schmid,F.; Ress,K.M.; Krämer,B.K.; Mayer,F.; Liebich,H.M.; Risler,T.; Seipel,L. Quantification of cibenzoline and its imidazole metabolite by high-performance liquid chromatography in human serum, *J.Chromatogr.*, **1990**, *528*, 267–273.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 222

CHROMATOGRAM

Retention time: 5.69
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: 1 mL Plasma or urine + 50 μL 2 μg/mL IS in MeCN + 2 mL buffer, vortex, add 2.5 mL benzene (CAUTION! Benzene is a carcinogen!), shake on a reciprocating shaker at 80-100 strokes/min for 10 min, centrifuge at 1460 g at 10° for 10 min. Remove 2 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 65°, reconstitute the residue in 400 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 7-8 μm Zorbax SCX

Mobile phase: MeCN:buffer 80:20 (Prepare buffer from 6 mL 1 M pH 6.0 phosphate buffer and 394 mL water. Prepare 1 M pH 6.0 phosphate buffer from 430 mL 1 M orthophosphoric acid + 570 mL 1 M KH_2PO_4, adjust pH to 6.0.) (Flush column with MeCN:water 80:20 at the end of each day.)

Flow rate: 1.5

Injection volume: 50

Detector: UV 214

CHROMATOGRAM

Retention time: 5.5

Internal standard: 2-[2,2-bis(4-methylphenyl)-1-cyclopropyl]-4,5-dihydro-1H-imidazole (4.5)

Limit of detection: 10 ng/mL (plasma)

Limit of quantitation: 50 ng/mL (urine)

OTHER SUBSTANCES

Noninterfering: metabolites

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Hackman,M.R.; Lee,T.L.; Brooks,M.A. Determination of cibenzoline in plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *273*, 347–356.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM
Retention time: 13.143

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 50 μL aliquot of a solution in MeOH:triethylamine 99:1 with 20 μL 0.1% NAPIC in dry toluene, vortex briefly, let stand at room temperature in the dark for 30 min, add 50 μL 1% ethanolamine in MeOH, let stand at room temperature for 15 min, evaporate to dryness under reduced pressure, reconstitute with 100 μL mobile phase, sonicate for 30 s, inject a 20 μL aliquot. (NAPIC is (-)-(S)-naproxen isocyanate; synthesis is as follows (protect from light). Dissolve 1 g (+)-(S)-naproxen in 30 mL acetone, cool to 0°, add a solution of 700 μL triethylamine in 2 mL acetone dropwise, add a solution of 450 μL ethyl chloroformate in 2 mL acetone dropwise, stir at 0° for 15 min, add a solution of 310 mg sodium azide in 1 mL water dropwise (Caution! Sodium azide is highly toxic!), stir for 1 h, pour into 60 mL ice water, stir for 10 min, filter, wash the solid with two 50 mL aliquots of ice-water, dry under reduced pressure to obtain flunoxaprofen azide. Dissolve 100 mg flunoxaprofen azide in 3 mL dry toluene, reflux for 10-15 min, cool to room temperature, filter. Evaporate the filtrate to dryness under reduced pressure and dry under reduced pressure to obtain NAPIC as an oil that crystallized in the desiccator (mp 48°), store in a desiccator under reduced pressure.)

HPLC VARIABLES
Column: 250 × 4.6 7 μm Nucleosil phenyl
Mobile phase: MeOH:water:diethylamine 63:37:0.05
Flow rate: 1
Injection volume: 20
Detector: F ex 276 em 356

CHROMATOGRAM
Retention time: 15.1 (S), 20.6 (R)

KEY WORDS
derivatization; chiral

REFERENCE
Martin,E.; Quinke,K.; Spahn,H.; Mutschler,E. (-)-(S)-Flunoxaprofen and (-)-(S)-naproxen isocyanate: two new fluorescent chiral derivatizing agents for an enantiospecific determination of primary and secondary amines, *Chirality*, **1989**, *1*, 223–234.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 50 μL aliquot of a solution in MeOH:triethylamine 99:1 with 20 μL 0.1% FLOPIC in dry toluene, vortex briefly, let stand at room temperature in the dark for 30 min, add 50 μL 1% ethanolamine in MeOH, let stand at room temperature for 15 min, evaporate to dryness under reduced pressure, reconstitute with 100 μL mobile phase, sonicate for 30 s, inject a 20 μL aliquot. (FLOPIC is (-)-(S)-flunoxaprofen isocyanate; synthesis is as follows. Dissolve 1 g (+)-(S)-flunoxaprofen in 30 mL acetone, cool to 0°, add a solution of 500 μL triethylamine in 2 mL acetone dropwise, add a solution of 370 μL ethyl chloroformate in 2 mL acetone dropwise, stir at 0° for 15 min, add a solution

of 250 mg sodium azide in 1 mL water dropwise (Caution! Sodium azide is highly toxic!), stir for 1 h, pour into 60 mL ice water, stir for 10 min, filter, wash the solid with two 50 mL aliquots of ice-water, dry under reduced pressure to obtain flunoxaprofen azide. Dissolve 100 mg flunoxaprofen azide in 3 mL dry toluene, reflux for 10-15 min, cool to room temperature, filter. Evaporate the filtrate to dryness under reduced pressure and dry under reduced pressure to obtain FLOPIC as a crystalline solid (mp 93-94°), store in a desiccator under reduced pressure.)

HPLC VARIABLES

Column: 250 × 4.6 7 μm Nucleosil phenyl (A) or 200 × 4.6 5 μm Nucleosil cyano (B)
Mobile phase: MeOH:80 mM NaCl in water 68:32 (A) or n-hexane:isopropanol 88:12 (B)
Flow rate: 1
Injection volume: 20
Detector: F ex 296 em 356

CHROMATOGRAM

Retention time: 18.4 (R (A)), 22.4 (S (A)), 15.9 (R (B)), 18.6 (S (B))

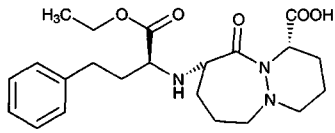
KEY WORDS

derivatization; chiral

REFERENCE

Martin,E.; Quinke,K.; Spahn,H.; Mutschler,E. (-)-(S)-Flunoxaprofen and (-)-(S)-naproxen isocyanate: two new fluorescent chiral derivatizing agents for an enantiospecific determination of primary and secondary amines, *Chirality*, **1989**, *1*, 223–234.

Cilazapril



Molecular formula: $C_{22}H_{31}N_3O_5.H_2O$
Molecular weight: 435.52
CAS Registry No.: 92077-78-6, 88768-40-5 (anhydrous)
Merck Index: 2332
Lednicer No.: 4 170

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 14.367

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: formulations
Sample preparation: Grind tablets to a fine powder. Weigh out amount equivalent to 25 mg cilazapril, extract with MeOH, filter. Mix 400-2000 μL filtrate with 500 μL 4 mg/mL IS in MeOH, make up to 10 mL with MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 10 μm LiChrosorb RP-18
Mobile phase: MeCN:buffer 30:70 (Buffer was 67 mM KH_2PO_4 adjusted to pH 2.4 with phosphoric acid.)
Flow rate: 1
Injection volume: 20

Detector: UV 211

CHROMATOGRAM
Retention time: 17.61
Internal standard: enalapril (8.58)
Limit of detection: 10 μg/mL
Limit of quantitation: 40 μg/mL

OTHER SUBSTANCES
Simultaneous: benazepril

KEY WORDS
tablets

REFERENCE
Gumieniczek,A.; Przyborowski,L. Determination of benazepril and cilazapril in pharmaceuticals by high performance liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2135–2142.

SAMPLE
Matrix: solutions

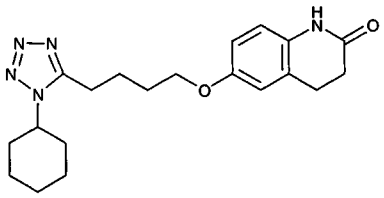
HPLC VARIABLES
Column: 250 × 4.6 Spherisorb 5 ODS-2
Mobile phase: n-Propanol:buffer 20:80 (Buffer was pH 3.0 phosphate buffer containing 0.4% triethylamine.)
Flow rate: 1
Detector: UV 240

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: quinapril, captopril, enalapril, benzepril, ramipril

REFERENCE
Barbato,F.; Morrica,P.; Quaglia,F. Analysis of ACE inhibitor drugs by high performance liquid chromatography, *Farmaco*, **1994**, *49*, 457–460.

Cilostazol



Molecular formula: $C_{20}H_{27}N_5O_2$
Molecular weight: 369.47
CAS Registry No.: 73963-72-1
Merck Index: 2335

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 10 μL 60 μg/mL IS in MeOH + 4 mL MeCN, vortex, centrifuge at 1700 g for 10 min. Remove the supernatant and evaporate the MeCN under a stream of air, reconstitute the residue in 1 mL 200 mM NaOH, add 5 mL chloroform, shake, centrifuge at 1700 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air, reconstitute the residue in 1 mL 200 mM NaOH, add 5 mL diethyl ether, shake, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL MeOH, inject a 40 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water 42:58
Flow rate: 1.7
Injection volume: 40
Detector: UV 254

CHROMATOGRAM

Retention time: 7.5
Internal standard: 6-[4-(1-cyclohexyl-1H-tetrazol-5-yl)propoxy]-3,4-dihydro-1-ethyl-2(1H)-quinolinone (OPC-13012) (12)
Limit of quantitation: 25 ng/mL

KEY WORDS

plasma; pharmacokinetics; human; dog (Arzneimittelforschung 1985; 35; 1124)

REFERENCE

Akiyama,H.; Kudo,S.; Odomi,M.; Shimizu,T. High-performance liquid chromatographic procedure for the determination of a new antithrombotic and vasodilating agent, cilostazol, in human plasma, *J.Chromatogr.*, **1985**, *338*, 456–459.

SAMPLE

Matrix: blood
Sample preparation: Rat. 1 mL Plasma + 10 μL 20 μg/mL IS in MeOH + 3 mL EtOH, vortex, centrifuge at 1700 g for 10 min. Remove the supernatant and evaporate the EtOH under a stream of air, reconstitute the residue in 1 mL 200 mM NaOH, add 5 mL ethyl ether, shake, centrifuge at 1700 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air, reconstitute the residue in 100 μL MeOH, inject a 40 μL aliquot. Rabbit. 500 μL Plasma + 5 μL 20 μg/mL IS in MeOH + 500 μL water + 3 mL EtOH, vortex, centrifuge at 1700 g for 10 min. Remove the supernatant and evaporate the EtOH under a stream of air, reconstitute the residue in 1 mL 200 mM NaOH, add 5 mL ethyl ether, shake, centrifuge at 1700 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air, reconstitute the residue in 100 μL MeOH, inject a 40 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water 42:58

Flow rate: 1.7
Injection volume: 40
Detector: UV 254

CHROMATOGRAM

Internal standard: 6-[4-(1-cyclohexyl-1H-tetrazol-5-yl)propoxy]-3,4-dihydro-1-ethyl-2(1H)-quinolinone (OPC-13012)
Limit of quantitation: 25 ng/mL

KEY WORDS

rat; rabbit; plasma; pharmacokinetics

REFERENCE

Akiyama,H.; Kudo,S.; Shimizu,T. The absorption, distribution and excretion of a new antithrombotic and vasodilating agent, cilostazol, in rat, rabbit, dog and man, *Arzneimittelforschung*, **1985**, *35*, 1124–1132.

SAMPLE

Matrix: bulk
Sample preparation: Dissolve 50 mg in 50 mL MeOH:water 50:50. Remove a 1 mL aliquot and add it to 1 mL 0.05% benzophenone in EtOH, make up to 10 mL with MeOH:water 50:50, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM potassium nitrate 50:50
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Internal standard: benzophenone

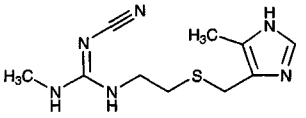
OTHER SUBSTANCES

Simultaneous: degradation products

REFERENCE

Shimizu,T.; Osumi,T.; Niimi,K.; Nakagawa,K. Physico-chemical properties and stability of cilostazol, *Arzneimittelforschung*, **1985**, *35*, 1117–1123.

Cimetidine



Molecular formula: $C_{10}H_{16}N_6S$
Molecular weight: 252.34
CAS Registry No.: 51481-61-9, 70059-30-2 (HCl)
Merck Index: 2337
Lednicer No.: 2 253; 4 89, 95, 112

SAMPLE

Matrix: blood, CSF, tissue
Sample preparation: Plasma. 25 μL Plasma + 100 μL 5 M NaOH + 5 mL dichloromethane, shake for 10 min, centrifuge at 1650 g for 10 min. Evaporate 4 mL aliquot of the organic phase. Dissolve the residue in 100 μL mobile phase. Inject a 25 μL aliquot. Tissue. Homogenize brain tissue with 1 mL saline on ice for 1 min. Add 100 μL 1 M NaOH, extract with 5 mL dichloromethane. Evaporate a 3 mL aliquot of the organic phase. Dissolve the residue in 100 μL mobile phase, centrifuge at 10000 g. Inject a 25 μL aliquot. CSF. Inject a 25 μL aliquot of the CSF directly.

HPLC VARIABLES

Column: 250 × 4 Senshu gel 5C18H (Senshu, Japan)
Mobile phase: MeCN:5 mM NaH_2PO_4 containing 5 mM tetramethylammonium chloride 5:95
Column temperature: 40
Flow rate: 2
Injection volume: 25
Detector: UV 320

CHROMATOGRAM

Internal standard: cimetidine

OTHER SUBSTANCES

Extracted: ranitidine

KEY WORDS

plasma; brain; rat; cimetidine is IS

REFERENCE

Nakada,Y.; Yamamoto,K.; Kawakami,J.; Sawada,Y.; Iga,T. Effect of renal failure on neurotoxicity of ranitidine in rats, *Biol.Pharm.Bull.*, **1996**, *19*, 323–325.

SAMPLE

Matrix: blood, tissue
Sample preparation: Plasma. 100 μL Plasma + 100 μL 75 μM IS + 100 μL 5 M NaOH + 5 mL dichloromethane, shake for 10 min, centrifuge at 2000 rpm for 10 min. Evaporate 4 mL of the organic phase to dryness under a stream of nitrogen. Dissolve residue in 400 μL mobile phase, inject a 20 μL aliquot. Tissue. Homogenize 500 mg liver with saline on ice for 1 min. Add 100 μL 75 μM IS, 100 μL 0.5 M NaOH, and 5 mL dichloromethane, shake for 10 min, centrifuge at 3000 rpm for 10 min. Evaporate 3 mL of the organic phase to dryness under a stream of nitrogen. Dissolve residue in 400 μL mobile phase, pass through a Ministar-RC 15 cartridge (Sartorius, Germany), inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 Senshu Pak ODS-1031 (Senshu Sciences, Japan)
Column: 250 × 4.6 Senshu Pak ODS -1251 (Senshu Sciences, Japan)

Mobile phase: MeCN:water 5:95 containing 5 mM NaH_2PO_4 and 5 mM tetramethylammonium chloride
Flow rate: 1.5
Injection volume: 20
Detector: UV 228

CHROMATOGRAM
Internal standard: nizatidine
Limit of detection: 50-100 μg/mL (sic)

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Takedomi,S.; Matsuo,H.; Yamano,K.; Yamamoto,K.; Iga,T.; Sawada,Y. Quantitative prediction of the interaction of midazolam and histamine H_2 receptor antagonists in rats, *Drug Metab.Dispos.*, **1998**, *26*, 318–323.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.602

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4.6 5 μm C18
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 1.5
Injection volume: 20
Detector: UV 228

CHROMATOGRAM
Retention time: 2.71

OTHER SUBSTANCES
Simultaneous: cisplatin (UV 198), dacarbazine (UV 300), granisetron (UV 300)

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeOH:water containing 0.03% phosphoric acid 20:80
Flow rate: 1
Detector: UV 201

REFERENCE
Walter,E.; Janich,S.; Roessler,B.J.; Hilfinger,J.M.; Amidon,G.L. HT29-MTX/Caco-2 cocultures as an in vitro model for the intestinal epithelium: In vitro-in vivo correlation with permeability data from rats and humans, *J.Pharm.Sci.*, **1996**, *85*, 1070–1076.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve a sample in MeOH to a concentration of about 1 mg/mL, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Spherisorb SCX
Mobile phase: MeOH:water 80:20 containing 20 mM ammonium formate and 2.3 mL/L trifluoroacetic acid
Flow rate: 1
Injection volume: 1-10
Detector: UV 270

CHROMATOGRAM
Retention time: 6.7

OTHER SUBSTANCES
Simultaneous: clomipramine, halofantrine, haloperidol, minoxidil, reserpine, verapamil

REFERENCE
Law,N.; Appleby,J.R.G. Re-evaluation of strong cation-exchange high-performance liquid chromatography for the analysis of basic drugs, *J.Chromatogr.A*, **1996**, *725*, 335–341.

SAMPLE
Matrix: solutions

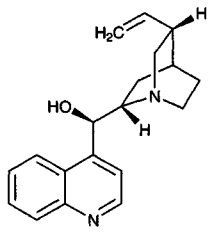
HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil ODS1
Mobile phase: MeOH:50 mM pH 3.0 phosphoric acid 10:90
Column temperature: 30
Flow rate: 1.5
Detector: radioactivity detection

OTHER SUBSTANCES
Also analyzed: atenolol, hydrochlorothiazide, ranitidine

KEY WORDS
tritium labeled

REFERENCE
Collett,A.; Sims,E.; Walker,D.; He,Y.-L.; Ayrton,J.; Rowland,M.; Warhurst,G. Comparison of HT29-18-C_1 and Caco-2 cell lines as models for studying intestinal paracellular drug absorption, *Pharm.Res.*, **1996**, *13*, 216–221.

Cinchonidine



Molecular formula: $C_{19}H_{22}N_2O$
Molecular weight: 294.40
CAS Registry No.: 485-71-2
Merck Index: 2345

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 3.6

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, prom-

azine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

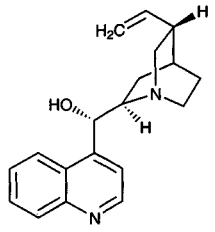
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazo-

cine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Cinchonine



Molecular formula: $C_{19}H_{22}N_2O$
Molecular weight: 294.40
CAS Registry No.: 118-10-5
Merck Index: 2346

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 202.8

CHROMATOGRAM

Retention time: 10.198

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

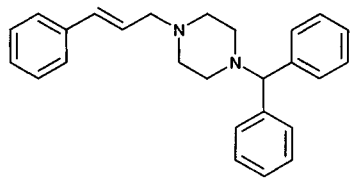
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Cinnarizine



Molecular formula: $C_{26}H_{28}N_2$
Molecular weight: 368.52
CAS Registry No.: 298-57-7
Merck Index: 2365
Lednicer No.: 1 58

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A: B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 19.258

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.6

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 177.82

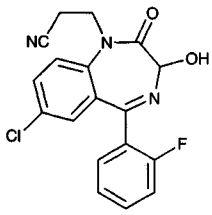
OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Cinolazepam



Molecular formula: $C_{18}H_{13}ClFN_3O_2$
Molecular weight: 357.77
CAS Registry No.: 75696-02-5
Merck Index: 2368

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: not given
Mobile phase: MeCN:buffer 44:56 (Buffer was MeCN:50 mM pH 2.5 sodium heptanesulfonate 5:95.)
Detector: UV 230

KEY WORDS
tablets

REFERENCE
Oelschläger,H.; Volke,J.; Belal,F. Analysis of drugs by polarography, XXXV: The polarographic behaviour of cinolazepam [1-(2-cyanoethyl)-7-chloro-3-hydroxy-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one] and assay of its tablets, *Arch.Pharm.(Weinheim)*, **1992**, *325*, 65–68.

SAMPLE
Matrix: urine
Sample preparation: Centrifuge urine, inject an alaiquot.

HPLC VARIABLES
Column: 250 × 4 7 μm LiChrosorb RP-18
Mobile phase: MeCN:10 mM orthophosphoric acid 30:70
Detector: UV 230

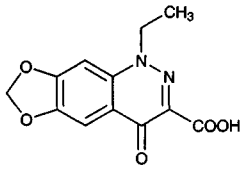
CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Extracted: oxazepam, glucuronides

KEY WORDS
human; rabbit

REFERENCE
Mascher,H.; Nitsche,V.; Schütz,H. Separation, isolation and identification of optical isomers of 1, 4-benzodiazepine glucuronides from biological fluids by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *306*, 231–239.

Cinoxacin



Molecular formula: $C_{12}H_{10}N_2O_5$
Molecular weight: 262.22
CAS Registry No.: 28657-80-9
Merck Index: 2369
Lednicer No.: 2 388

SAMPLE

Matrix: blood
Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 250 × 4.6 Spherisorb ODS-2 endcapped
Mobile phase: MeCN:buffer 20:80 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)
Column temperature: 37
Flow rate: 1
Detector: UV 268

CHROMATOGRAM

Retention time: 9.88
Internal standard: rosoxacin (5.79)

KEY WORDS

plasma; ultrafiltrate

REFERENCE

Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

SAMPLE

Matrix: urine
Sample preparation: Make up 1 mL urine to 25 mL with deionized water. Adjust to pH 2.5-3 with HCl, extract with 25 mL chloroform. Separate the organic layer, dry the organic phase with sodium sulfate, evaporate it to dryness. Dissolve the residue in 3 mL MeCN, dilute to 10 mL with water, filter (0.45 μm). Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeCN:0.4 mM oxalic acid in water 28:72
Flow rate: 2.0
Injection volume: 20
Detector: F ex 270 em 440

CHROMATOGRAM

Retention time: 1.64
Limit of detection: 2.05 ng/mL

OTHER SUBSTANCES

Extracted: oxolinic acid, pipemidic acid

REFERENCE

Durán Merá,I.; Galeano Díaz,T.; Rodríguez Cáceres,M.I.; Salinas López,F. Determination of the chemotherapeutic quinolonic and cinolonic derivatives in urine by high-performance liquid chromatography with ultraviolet and fluorescence detection in series, *J.Chromatogr.A*, **1997**, *787*, 119–127.

SAMPLE

Matrix: urine

Sample preparation: Make up 1 mL urine to 25 mL with mobile phase, filter (0.45 μm). Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 Nova-Pak C18

Mobile phase: MeCN:400 μM oxalic acid in water 28:72

Flow rate: 2.0

Injection volume: 20

Detector: UV 265

CHROMATOGRAM

Retention time: 1.64

Limit of detection: 700 ng/mL

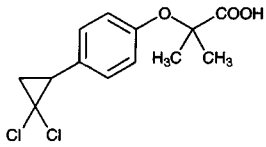
OTHER SUBSTANCES

Simultaneous: nalidixic acid, oxolinic acid, pipemidic acid, piromidic acid

REFERENCE

Durán Merá,I.; Galeano Díaz,T.; Rodríguez Cáceres,M.I.; Salinas López,F. Determination of the chemotherapeutic quinolonic and cinolonic derivatives in urine by high-performance liquid chromatography with ultraviolet and fluorescence detection in series, *J.Chromatogr.A*, **1997**, *787*, 119–127.

Ciprofibrate



Molecular formula: $C_{13}H_{14}Cl_2O_3$
Molecular weight: 289.16
CAS Registry No.: 52214-84-3
Merck Index: 2373
Lednicer No.: 3 44

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 50 µL 100 µg/mL IS in ethyl acetate + 2 mL 1 M HCl + 200 µL 60% perchloric acid, extract twice with 10 mL hexane. Combine the organic layers and evaporate them to dryness under a stream of nitrogen using a warm water bath, reconstitute the residue in 2 mL MeCN, add 2 mL hexane, shake vigorously. Remove the MeCN layer and evaporate it to dryness under a stream of nitrogen using a warm water bath, reconstitute the residue in 500 µL MeCN:THF 10:1, add 500 µL 100 mM pH 4 K_2HPO_4, shake vigorously, inject a 100 µL aliquot.

HPLC VARIABLES

Guard column: 25 × 3.9 37-50 µm Bondapak phenyl/Corasil
Column: 300 × 3.9 10 µm C2-phenyl (Waters)
Mobile phase: MeCN:THF:100 mM pH 4 K_2HPO_4 96:10:104
Flow rate: 2
Injection volume: 100
Detector: UV 232

CHROMATOGRAM

Retention time: 4
Internal standard: 2-[4-(2,2-dichloro-3-phenylcyclopropyl)phenoxy]-2-methylpropanoic acid (6)
Limit of quantitation: 690 ng/mL

KEY WORDS

plasma; human; rat

REFERENCE

Park,G.B.; Biddlecome,C.E.; Koblantz,C.; Edelson,J. Determination of ciprofibrate in human plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1982**, *227*, 534–539.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 21.22

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 200 μL Microsomal incubation + 800 μL chloroform:MeOH 2:1, agitate vigorously for 1 min, centrifuge, inject a 5 μL aliquot of the upper water/MeOH layer.

HPLC VARIABLES
Column: 300 × 4 μBondapak C18
Mobile phase: MeOH:water 55:45 containing 9 mM KH_2PO_4, pH 5.5
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Extracted: ATP, metabolites

KEY WORDS
rat; liver

REFERENCE
Bronfman,M.; Amigo,L.; Morales,M.N. Activation of hypolipidaemic drugs to acyl-coenzyme A thioesters, *Biochem.J.*, **1986**, *239*, 781–784.

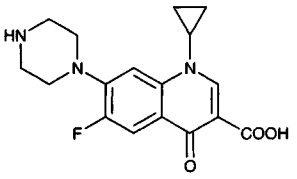
SAMPLE
Matrix: urine
Sample preparation: Adjust pH of 1 mL urine to 12 with NaOH, heat at 40° for 30 min, acidify to pH 3.0 extract twice with 5 mL n-hexane. Evaporate the combined extracts, reconstitute in 1 mL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil 120 C18
Mobile phase: MeCN:5 mM pH 3.3 PIC B7 (heptanesulfonic acid) 50:50
Flow rate: 1
Detector: UV 230

REFERENCE

Oelschläger,H.; Rothley,D.; Hellwich,K.-H.; Schmidt,W. Zur Pharmakokinetik von Lipidsenkern, 6. Mitt. Ist 2-(4-(2,2-Dichlorocyclopropyl)-phenoxy)-propan ein Metabolit des lipidsenkers Ciprofibrat? [The pharmacokinetics of hypolipidemic agents, VI: Is 2-(4-(2,2-dichlorocyclopropyl)-phenoxy)-propane a metabolite of the hypolipidemic agent ciprofibrate?], *Arch.Pharm.(Weinheim)*, **1989**, *322*, 629–632.

Ciprofloxacin



Molecular formula: $C_{17}H_{18}FN_3O_3$
Molecular weight: 331.35
CAS Registry No.: 85721-33-1, 86393-32-0 (hydrochloride monohydrate)
Merck Index: 2374
Lednicer No.: 4 141

SAMPLE

Matrix: aqueous humor
Sample preparation: Add 350 μL distilled water and 1 μg/mL pipemidic acid solution to 50 μL aqueous humor, vortex for 30 s, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: Guard-Pak with Novapak C18 insert
Column: 100 × 8.0 4 μm Novapak C18
Mobile phase: MeCN:MeOH:400 mM citric acid 7.14:21.43:71.43
Flow rate: 1
Injection volume: 20
Detector: F ex 278 em 450

CHROMATOGRAM

Retention time: 7.52
Internal standard: pipemidic acid (4.88)
Limit of detection: 250 pg/mL

REFERENCE

Basci,N.E.; Bozkurt,A.; Kalayci,D.; Kayaalp,S.O. Rapid liquid chromatographic assay of ciprofloxacin in human aqueous humor, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 353–356.

SAMPLE

Matrix: aqueous humor, blood
Sample preparation: Aqueous humor. Inject a 10 μL aliquot directly. Plasma. Condition a 3 mL C18 SPE cartridge (Varian) with two 3 mL portions of MeCN and 3 mL buffer. Add 2 mL buffer to 500 μL of plasma, mix, add to the SPE cartridge. Wash with 3 mL buffer. Remove moisture with vacuum (200 mbar) for 10 min. Elute with two 500 μL portions of MeCN:buffer 40:60. Vortex the eluate, inject a 10 μL aliquot. (Buffer was 100 mM Tris adjusted to pH 5.0 with HCl).

HPLC VARIABLES

Column: 300 × 4.6 5 μm endcapped ODS-Hypersil
Mobile phase: MeCN:DMF:10 mM NaH_2PO_4 15:6:79, adjusted to pH 3.0 with 85% phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 285

CHROMATOGRAM

Retention time: 12.0
Internal standard: ciprofloxacin
Limit of detection: 80 ng/mL (aqueous humor), 310 ng/mL (plasma)

OTHER SUBSTANCES

Extracted: cefotaxime, ofloxacin

KEY WORDS
plasma; SPE; ciprofloxacin is IS

REFERENCE
Kraemer,H.-J.; Gehrke,R.; Breithaupt,A.; Breithaupt,H. Simultaneous quantification of cefotaxime, desacetylcefotaxime, ofloxacine and ciprofloxacine in ocular aqueous humor and in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *700*, 147–153.

SAMPLE
Matrix: blood
Sample preparation: Extract plasma sample with dichloromethane:n-butanol 95:5 and dry the solution. Treat organic layer residue with Moscher's acid chloride + triethanolamine in dichloromethane medium for 1 hr. Reconstitute product. Inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.2 5μm ODS
Mobile phase: MeCN:0.2% phosphoric acid 7:3
Column temperature: 35
Detector: F ex 290 em 470

CHROMATOGRAM
Retention time: 7.4
Internal standard: ciprofloxacin

OTHER SUBSTANCES
Extracted: grepafloxacin

KEY WORDS
ciprofloxacin is IS; derivatization; plasma

REFERENCE
Tata,P.N.V.; Bramer,S.L. Enantiomeric assay of grepafloxacin in plasma (Abstract 4162), *Pharm.Res.*, **1997**, *14*, S684–S684.

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL blood with 1 mL 0.25 mM Triton, vortex for 30 s, add 4 mL 6% trichloroacetic acid. Vortex for 30 s, centrifuge at 2000 g for 10 min, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm C18
Mobile phase: MeCN:buffer 10:90 (Buffer was 1 L 25 mM phosphoric acid and 15 mL 40% tetrabutylammonium hydrogen sulfate adjusted to pH 3.0 with 66.6 mM phosphate buffer.)
Flow rate: 2
Injection volume: 100
Detector: F ex 330 em 450

CHROMATOGRAM
Retention time: 9
Limit of detection: 30 ng/mL (plasma), 50 ng/mL (blood)

KEY WORDS
plasma; pharmacokinetics; rabbit

REFERENCE
Colino,C.I.; García Turiño,A.; Sanchez Navarro,A.; Lanao,J.M. A comparative study of ofloxacin and ciprofloxacin erythrocyte distribution, *Biopharm.Drug Dispos.*, **1998**, *19*, 71–77.

SAMPLE
Matrix: blood
Sample preparation: Add 2 mL pH 7.5 phosphate buffer to 250 μL plasma. Extract twice with 5 mL portions of ethyl acetate. Evaporate the organic layer. Reconstitute the residue with mobile phase and inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Partisil C8
Mobile phase: MeCN:2 mM phosphoric acid:triethylamine 15:85:0.15, pH 3.5
Flow rate: 1
Detector: UV 308

CHROMATOGRAM
Retention time: 10
Internal standard: ciprofloxacin

OTHER SUBSTANCES
Extracted: sparfloxacin

KEY WORDS
plasma; ciprofloxacin is IS

REFERENCE
Bhatti,M.M.; Hanson,G.D. Determination of cisapride in human plasma by high-performance liquid chromatography with ultraviolet detection (Abstract 2504), *Pharm.Res.*, **1997**, *14*, S378–S378.

SAMPLE
Matrix: blood
Sample preparation: Add 20 μL 10 μg/mL IS in MeOH:0.1% trifluoroacetic acid 15:85 and 5 μL (sic) MeCN to 300 μL plasma. Centrifuge at 600 g for 10 min. Evaporate the supernatant under nitrogen at 40° for 30 min. Reconstitute the residue in 200 μL MeOH: 0.1% trifluoroacetic acid 15:85. Inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 12.5 × 4 Zorbax RX-C18
Column: 150 × 4.6 5 μm Zorbax SB-C8
Mobile phase: MeCN:water:trifluoroacetic acid 19:81:0.02
Flow rate: 1
Injection volume: 50
Detector: UV 279

CHROMATOGRAM
Retention time: 4.3-4.4
Internal standard: norfloxacin (3.8-3.9)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: enrofloxacin

KEY WORDS
cat; plasma

REFERENCE

Kordick,D.L.; Papich,M.G.; Breitschwerdt,E.B. Efficacy of enrofloxacin or doxycycline for treatment of Bartonella henselae or Bartonella clarridgeiae infection in cats, *Antimicrob.Agents Chemother.*, **1997**, *41*, 2448–2455.

SAMPLE

Matrix: blood

Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 250 × 4.6 Spherisorb ODS-2 endcapped

Mobile phase: MeCN:buffer 13:87 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)

Column temperature: 37

Flow rate: 1

Detector: UV 279

CHROMATOGRAM

Retention time: 9.71

Internal standard: enoxacin (6.47)

KEY WORDS

plasma; ultrafiltrate

REFERENCE

Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

SAMPLE

Matrix: blood

Sample preparation: 200 μL Plasma + 200 μL 100 mM phosphoric acid, vortex, centrifuge at 3500 g for 10 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Guard column: 17 × 4.4 5 μm Nucleosil 100 RP18

Column: 250 × 4.6 5 μm Nucleosil 100 RP18

Mobile phase: Gradient. MeCN:buffer 11:89 for 6.2 min, 50:50 for 3.5 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 50

Flow rate: 1 for 6.2 min, 1.5 for 3.5 min, 1 for 7 min

Detector: F ex 277 em 455

CHROMATOGRAM

Retention time: 6

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; pharmacokinetics; comparison with capillary electrophoresis

REFERENCE
Bannefeld,K.-H.; Stass,H.; Blaschke,G. Capillary electrophoresis with laser-induced fluorescence detection, an adequate alternative to high-performance liquid chromatography, for the determination of ciprofloxacin and its metabolite desethyleneciprofloxacin in human plasma, *J.Chromatogr.B*, **1997**, *692*, 453–459.

SAMPLE
Matrix: blood, CSF, tissue
Sample preparation: Plasma, CSF. Mix 100 μL Plasma or CSF with 1.0 mL 100 mM phosphate buffer. Extract with 5 mL chloroform (Caution! Chloroform is a carcinogen!) containing 1.0% ethyl chloroformate by shaking with a reciprocal shaker for 10 min. Remove 4 mL organic phase, dry it with rotary evaporator at 40°, reconstitute the residue in 100 μL mobile phase and inject a 20 μL aliquot. Tissue. Homogenize brain sample in 100 mM pH 7.0 phosphate buffer 1:4. Extract 1 mL homogenate with 5 mL dichloromethane by shaking with a reciprocal shaker for 10 min. Remove 4 mL organic phase and back-extract with 4 mL 1 mM sodium hydroxide, extract the aqueous phase as described above for the plasma, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 6 Nucleosil 5C18
Mobile phase: MeOH:5 mM sodium lauryl sulfate 60:40, adjusted to pH 2.5 with phosphoric acid
Column temperature: 50
Flow rate: 1.2
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Internal standard: ciprofloxacin
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: enoxacin

KEY WORDS
plasma; brain; rat; derivatization; ciprofloxacin is IS

REFERENCE
Ohtani,H.; Noma,S.; Kawakami,J.; Sawada,Y.; Iga,T. Lack of potentiation with felbinac patch on the convulsive toxicity of enoxacin in rats, *Biol.Pharm.Bull.*, **1996**, *19*, 995–997.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. Mix 100 μL plasma with 900 μL 100 mM phosphate buffer, 100 μL 10 μg/mL IS and 5 mL chloroform:ethyl chloroformate 99:1, shake for 10 min, centrifuge at 1620 g for 5 min, evaporate the organic phase under reduced pressure, dissolve the residue in 100 μL MeOH:50 mM NaOH 2:1, inject a 20 μL aliquot. Tissue. Homogenate the cerebrum sample with 4 volumes of 100 mM phosphate buffer. Mix 1 mL homogenate with 100 μL 10 μg/mL IS and 5 mL dichloromethane, shake for 10 min, centrifuge at 1620 g for 5 min. Mix 4 mL 1 mM NaOH with 4 mL organic phase, shake for 10 min, centrifuge at 1620 g for 5 min, collect 3 mL aqueous phase and treat in a manner similar to that for the plasma sample, except for the IS addition. Inject a 20 μL aliquot. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil 5 C18
Mobile phase: MeOH:5 mM sodium dodecylsulfate adjusted to pH 2.5 with phosphoric acid

Flow rate: 0.8
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Internal standard: pipemidic acid

OTHER SUBSTANCES
Extracted: foscarnet
Simultaneous: enoxacin

KEY WORDS
plasma; brain; mouse; pharmacokinetics; derivatization

REFERENCE
Matsuo,H.; Ryu,M.; Nagata,A.; Uchida,T.; Kawakami,J.-I.; Yamamoto,K.; Iga,T.; Sawada,Y. Neurotoxicodynamics of the interaction between ciprofloxacin and foscarnet in mice, *Antimicrob.Agents Chemother.*, **1998**, *42*, 691–694.

SAMPLE
Matrix: blood, urine
Sample preparation: Mix 250 μL plasma or 10 μL urine with 250 μL phosphate buffer Add 4 mL dichloromethane, vortex for 30 s, centrifuge at 2000 g for 5 min. Remove the organic layer and evaporate it under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 10 μL aliquot. (Phosphate buffer was 70 mM KH_2PO_4:80 mM Na_2HPO_4 40:60.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeOH:5 mM copper (II) sulfate pentahydrate containing 10 mM L-isoleucine 12.5:87.5
Column temperature: 35
Flow rate: 1
Injection volume: 10
Detector: UV 330

CHROMATOGRAM
Retention time: 13
Internal standard: ciprofloxacin

OTHER SUBSTANCES
Extracted: levofloxacin

KEY WORDS
plasma; ciprofloxacin is IS

REFERENCE
Wong,F.A.; Juzwin,S.J.; Flor,S.C. Rapid stereospecific high-performance liquid chromatographic determination of levofloxacin in human plasma and urine, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 765–771.

SAMPLE
Matrix: blood, urine
Sample preparation: Blood. 500 μL Serum or plasma + 100 μL 20 μg/mL IS in 100 mM phosphoric acid + 300 μL MeCN:5 M trichloroacetic acid 50:50, vortex, add 100 μL MeCN, add 300 μL water, vortex, centrifuge at 1500 g for 15 min, inject a 10 μL aliquot of the supernatant. Urine. Dilute urine 1:20 (or more) with 50 mM pH 3.0 KH_2PO_4, remove a

500 μL aliquot and add it to 100 μL 20 μg/mL IS in 100 mM phosphoric acid, add 700 μL 100 mM trichloroacetic acid, vortex, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 5 × 3 PLRP-S (Polymer Laboratories)
Column: 150 × 4.6 PLRP-S (Polymer Laboratories)
Mobile phase: MeCN:MeOH:20 mM pH 3.0 trichloroacetic acid 22:4:74
Column temperature: 30
Flow rate: 0.7
Injection volume: 10
Detector: F ex 277 em 418 following post-column photolysis. The column effluent flowed through a 10 m × 0.25 mm knitted PTFE coil irradiated with a UV 254 low pressure lamp and flowed to the detector.

CHROMATOGRAM
Retention time: 8
Internal standard: 1-isopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid (13)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; plasma; post-column reaction; post-column photochemical derivatization

REFERENCE
Krol,G.J.; Beck,G.W.; Benham,T. HPLC analysis of ciprofloxacin and ciprofloxacin metabolites in body fluids, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 181–190.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 278.3

CHROMATOGRAM
Retention time: 9.102

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve a sample in mobile phase, sonicate, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:THF:buffer 5:10:85 (Buffer was 5 mM 1-hexanesulfonic acid adjusted to pH 3.0 with 100 mM phosphoric acid.)
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 11.5-15.0
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Lacroix,P.M.; Curran,N.M.; Sears,R.W. High-performance liquid chromatographic methods for ciprofloxacin hydrochloride and related compounds in raw materials, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 641–654.

SAMPLE
Matrix: growth medium
Sample preparation: 500 μL Growth medium + 500 μL 100 μg/mL IS in cold (4°) MeCN, vortex, centrifuge at 3000 g for 5 min. Remove a 500 μL aliquot of the supernatant, filter (0.45 μm Acrodisc syringe filter), inject a 30 μL aliquot. (Protect all specimens from light.)

HPLC VARIABLES
Guard column: C18 5U (Alltech)
Column: 150 × 4.6 7 μm Adsorbosphere HS C18 7U
Mobile phase: MeCN:20 mM pH 3.0 phosphate buffer 35:65 containing 0.2% triethylamine and 0.2% sodium dodecyl sulfate, adjusted to pH 3.0 with 85% phosphoric acid
Flow rate: 1.75
Injection volume: 30
Detector: UV 280

CHROMATOGRAM
Retention time: 4.67
Internal standard: sparfloxacin (7.09)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: clinafloxacin, levofloxacin, ofloxacin, sparfloxacin, temafloxacin, trovafloxacin

KEY WORDS
Mueller-Hinton broth

REFERENCE
Wright,D.H.; Herman,V.K.; Konstantinides,F.N.; Rotschafer,J.C. Determination of quinolone antibiotics in growth media by reversed-phase high-performance liquid chromatography, *J.Chromatogr.B*, **1998**, *709*, 97–104.

SAMPLE
Matrix: milk
Sample preparation: Condition a 500 mg Bond Elut LRC PRS SPE cartridge with 5 mL MeOH and 5 mL extracting solution 65:35. Add 25 mL extracting solution to 5 mL milk, shake for 15 s, add 4 g anhydrous sodium sulfate, shake for 15 s, centrifuge at 3000 rpm at 5° for 5 min. Remove the supernatant and repeat the extraction with 25 mL extracting solution as before except do not add any more sodium sulfate, mix mechanically, centrifuge, combine the supernatants, add 25 mL 1% acetic acid, shake for 10-15 s. Freeze for 30 min to facilitate precipitation, centrifuge at 2500 rpm at 5° for 10 min. Add 75 mL to the SPE cartridge, pass the entire sample through the cartridge, then add 2 mL MeOH, wash with 5 mL water, wash with 2 mL MeOH. Elute with 2.5 mL 25% ammonium hydroxide-MeOH. Evaporate to dryness under nitrogen at 55°, dissolve the residue in 2 mL 1% acetic acid, sonicate for 1 min, vortex for 20 s, filter (0.45 μm), inject an aliquot. (Extracting solution was 1% aqueous acetic acid:EtOH 1:99.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil
Mobile phase: MeCN:2% acetic acid 15:85
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: F ex 278 em 450, with a 418 nm cut-off filter

CHROMATOGRAM
Retention time: 3.1
Limit of detection: 0.4 ppb
Limit of quantitation: 5 ppb

OTHER SUBSTANCES
Extracted: enrofloxacin, difloxacin, sarafloxacin

KEY WORDS
SPE

REFERENCE
Roybal,J.E.; Pfenning,A.P.; Turnipseed,S.B.; Walker,C.C.; Hurlbut,J.A. Determination of four fluoroquinolones in milk by liquid chromatography, *J.AOAC Int.*, **1997**, *80*, 982–987.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: Asahipack ODP 50
Mobile phase: MeCN:water from 10:90 to 40:60 over 10 min
Detector: UV

OTHER SUBSTANCES
Simultaneous: sparfloxacin

REFERENCE
Rispal,P.; Grellet,J.; Celerier,C.; Breilh,D.; Dorian,M.; Pellegrin,J.L.; Saux,M.C.; Leng,B. Comparative uptake of sparfloxacin and ciprofloxacin into human THP 1 monocytic cells, *Arzneimittelforschung*, **1996**, *46*, 316–319.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 4 μm NovaPak C18
Mobile phase: MeCN:MeOH:buffer:acetic acid 2.5:10:86.5:1 containing 20 mM triethylamine (The pH 2.7 buffer was 0.4% diammonium hydrogen phosphate in water containing 0.4% (?) tetrabutylammonium hydrogen sulfate.)
Flow rate: 1
Detector: UV 279

CHROMATOGRAM
Retention time: 21.6

OTHER SUBSTANCES
Extracted: enrofloxacin, ofloxacin

REFERENCE
Cester,C.C.; Toutain,P.L. A comprehensive model for enrofloxacin to ciprofloxacin transformation and disposition in dog, *J.Pharm.Sci.*, **1997**, *86*, 1148–1155.

SAMPLE
Matrix: solutions
Sample preparation: Filter (0.45 μm) a solution in MeCN:water 10:90, inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: MeCN:buffer 7:93 (Buffer was 25 mM phosphoric acid adjusted to pH 3.89 with 100 mM tetrabutylammonium hydroxide.)
Flow rate: 1
Injection volume: 10
Detector: UV 280

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Simultaneous: enoxacin, fleroxacin, norfloxacin, ofloxacin (UV 295), pipemidic acid

REFERENCE
Barbosa,J.; Bergés,R.; Sanz-Nebot,V. Solvatochromic parameter values and pH in aqueous-organic mixtures used in liquid chromatography. Prediction of retention of a series of quinolones, *J.Chromatogr.A*, **1996**, *719*, 27–36.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 10 mL 500 mg Bond Elut LRC PRS SPE cartridge with 2 mL MeOH and 2 mL equilibrating solution. 2 g Catfish muscle + 18 mL extracting solution, homogenize for 20 s, centrifuge at 3000 rpm for 5 min, decant the supernatant. Add another 18 mL extracting solution to the pellet and homogenize again, centrifuge at 3000 rpm for 5 min, combine the supernatants. Add 20 mL 1% glacial acetic acid, freeze for 30 min, centrifuge at 2500 rpm at 4° for 10 min. Add the extracts to the SPE cartridge, wash with 2 mL MeOH, 5 mL water, and 2 mL MeOH. Let the SPE cartridge dry for 30 s. Elute with 2 mL MeOH:30% ammonium hydroxide 80:20, dry the eluate under nitrogen at 50°. Reconstitute the residue in 500 μL mobile phase, filter (0.45 μm), inject an aliquot.

(The extracting solution was EtOH:water:glacial acetic acid 98:1:1. The equilibrating solution was extracting solution:1% glacial acetic acid 35:20.)

HPLC VARIABLES

Column: 150 × 2 5 μm Inertsil Phenyl
Mobile phase: MeCN:2% formic acid 14:86
Column temperature: 40
Flow rate: 0.35
Injection volume: 50
Detector: MS, Hewlett-Packard 5989, Model 59987A electrospray, nitrogen drying gas 40 mL/min, 260°, nebulizing gas nitrogen, 80 psi, m/z 231

CHROMATOGRAM

Retention time: 3.74-3.84
Limit of detection: 10 ppb
Limit of quantitation: 20 ppb

OTHER SUBSTANCES

Extracted: enrofloxacin

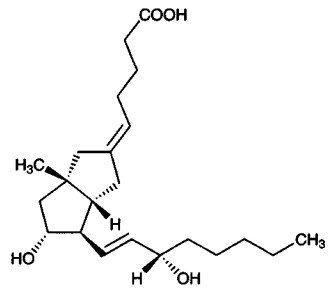
KEY WORDS

catfish; muscle; SPE

REFERENCE

Turnipseed,S.B.; Walker,C.C.; Roybal,J.E.; Pfenning,A.P.; Hurlbut,J.A. Confirmation of fluoroquinolones in catfish muscle by electrospray liquid chromatography/mass spectrometry, *J.AOAC Int.*, **1998**, *81*, 554–562.

Ciprostene



Molecular formula: $C_{22}H_{36}O_8$
Molecular weight: 727.04
CAS Registry No.: 81845-44-5, 81703-55-1 (calcium salt)
Lednicer No.: 4 14

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL 100 mg Bond Elut C2 SPE cartridge with two 1 mL portions of MeCN, with 1 mL water, and with 1 mL 1% phosphoric acid, do not allow to dry. Condition a Bond Elut CN SPE cartridge with two 1 mL portions of n-hexane. 250 μL Plasma + 50 μL 375 ng/mL carbacyclin in MeOH + 750 μL 1% phosphoric acid, add to the C2 SPE cartridge, wash with two 1 mL portions of water, wash with two 1 mL portions of MeOH:water 40:60, dry under vacuum for 10 min, wash with two 1 mL portions of n-hexane:MTBE 75:25, elute with 1 mL n-hexane:MTBE 20:80. Evaporate the eluate to dryness under reduced pressure, reconstitute with 100 μL MeOH, evaporate to dryness under a stream of air, add 10 mg solid potassium bicarbonate:sodium sulfate 1:1, add 50 μL 200 μM dibenzo-18-crown-6 in acetone, add 50 μL 1 mM 4-bromomethyl-7-acetoxycoumarin in acetone, stir at 50° for 30 min, cool, add 900 μL n-hexane, vortex, add to the CN SPE cartridge, wash with two 1 mL portions of n-hexane, wash with two 1 mL portions of n-hexane:ethyl acetate 80:20, dry under vacuum for 1 min, elute slowly with 1 mL MeCN. Evaporate the eluate to dryness under a stream of air at 30°, reconstitute the residue in 50 μL MeCN:water:trifluoroacetic acid 50:50:0.1, inject an aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 NewGuard RP18
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeCN:water:trifluoroacetic acid 55:44.9:0.1
Column temperature: 45
Flow rate: 1.5
Detector: F ex 370 em 466 following post-column reaction. The column effluent mixed with 100 mM NaOH pumped at 0.5 mL/min and the mixture flowed through a 4 mL knitted coil of PTFE tubing at 80° to the detector.

CHROMATOGRAM

Retention time: 31.3
Internal standard: carbacyclin (23.7)
Limit of quantitation: 5 ng/mL

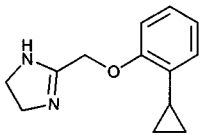
KEY WORDS

plasma; derivatization; post-column reaction; SPE

REFERENCE

James,C.A.; Simmonds,R.J.; Burton,N.K. An HPLC assay for a prostacyclin analogue, ciprostene calcium, in human plasma, *J.Liq.Chromatogr.*, **1990**, *13*, 1143–1158.

Cirazoline



Molecular formula: $C_{13}H_{16}N_2O$
Molecular weight: 216.28
CAS Registry No.: 59939-16-1

SAMPLE
Matrix: solutions

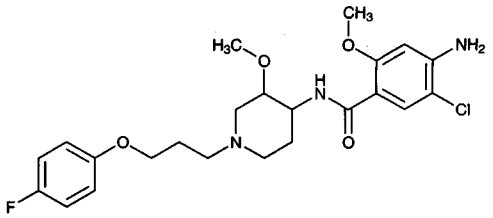
HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 8.71

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Cisapride



Molecular formula: $C_{23}H_{29}ClFN_3O_4$
Molecular weight: 465.95
CAS Registry No.: 81098-60-4
Merck Index: 2377

SAMPLE

Matrix: blood
Sample preparation: Add IS to 1 mL plasma, extract under basic conditions with 5 mL n-butyl chloride. Evaporate the organic layer to dryness under nitrogen. Dissolve the residue in 200 μL mobile phase and inject an aliquot.

HPLC VARIABLES

Column: 150 × 2 Hypersil-ODS
Mobile phase: MeCN:water:triethylamine 37:62.9:0.1
Detector: UV 276

CHROMATOGRAM

Retention time: 4.6
Internal standard: R054680 (10.1)
Limit of quantitation: 2 ng/mL

KEY WORDS

plasma

REFERENCE

Sirisuth,N.; Joubert,A.E.; Eddington,N.D. Development and validation of an HPLC method for ADD 196022, a member of the new class of enaminone anticonvulsants (Abstract 2496), *Pharm.Res.*, **1997**, *14*, S376–S376.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 214.6

CHROMATOGRAM

Retention time: 14.627

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.